

COMPARATIVE EVALUATION OF FOUR PURIFIED DIETARY PROTEINS FOR THE JUVENILE *PENAEUS INDICUS*

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ABSTRACT

Four purified proteins, albumen (egg), casein, fibrin (blood), and gelatin, were evaluated for the juvenile penaeid prawn *Penaeus indicus* (24.5 mm in length and 63 mg in weight) and compared in terms of their true digestibility (TD), net protein utilization (NPU), protein efficiency ratio (PER), and biological value (BV) in conjunction with growth and food conversion ratio (FCR). The metabolic faecal nitrogen (MFN) was found to be 324 mg N per 100 g diet consumed. Among the purified proteins tested, gelatin showed high TD of 93.4% ($p < 0.05$) followed by fibrin (82.4%), casein (76.4%), and albumen (72.5%). However, fibrin and albumen recorded high NPU and BV, gelatin showing poor results. The albumen diet gave the highest growth and PER ($p < 0.01$) and low FCR and emerged as the best protein source for juvenile *P. indicus*.

The BV and NPU increased with dietary protein up to 33.3% in albumen diets and 29.3% in casein diets and declined with further increase in the protein level. However, TD tended to be higher at lower dietary protein levels in both albumen and casein diets. The excretion of nitrogen in faeces increased with dietary protein and reached a peak at 37% protein in the diet. It rapidly declined with further increase in the dietary protein. The excretion of faecal nitrogen was higher in prawns fed with albumen diets than in those fed with casein diets. The prawns showed a dietary protein requirement of 25% with albumen diets and 29.3% with casein diets. The determination of nitrogen balance indicated the actual needs of nitrogen for zero nitrogen balance and explained the low protein requirement of the prawns. The application of this approach along with other parameters in determining the dietary protein requirement more accurately for aquatic animals is discussed.

Protein requirement of penaeid prawns as reported in the literature varies widely not only in different species but also in individual species at different life stages (New, 1976). These variations are attributed mainly to different sources of proteins used for formulating the diets (Kanazawa, 1984), the amino acid profile of the protein used, the age of the test animals, and the availability of other energy-giving nutrients such as carbohydrate and lipid in the diet. The primary factor, however, seems to be selection of appropriate protein source used in the dietary

requirement studies. In this context, it is felt that a comparative evaluation of purified proteins, which are generally used for formulation of diets, is essential. In the present study four purified proteins, albumen (egg), casein, fibrin (blood), and gelatin, were evaluated for formulation of the feed of the prawn *Penaeus indicus*; the metabolic faecal nitrogen determined and the influence of dietary protein in relation to the source on different nutritional parameters were discussed.

MATERIALS AND METHODS

Formulation and preparation of diets

Four purified proteins, albumen (egg, BDH), casein (fat free, SISCO), fibrin (blood, SIGMA) and gelatin (BDH), were selected for comparative evaluation. With each one of them as a protein source, four purified diets PE₁, PE₂, PE₃, and PE₄ and zero protein diet PE₀, containing no protein, were formulated. The composition of the diets is given in Table 1. In another experiment two sets of five diets each having 20% to 60% of albumen and casein were formulated to study and compare the effect of protein level in the diet on its digestibility, net protein utilization (NPU), biological value (BV), protein efficiency ratio (PER), and growth. The composition of these diets is presented in Table 2.

Table 1. Composition of the purified diets PE₀ to PE₄

Ingredients	PE ₀	PE ₁	PE ₂	PE ₃	PE ₄
Albumen (egg)	0.00	40.0			
Casein (fat free)	0.00		40.0		
Fibrin	0.00			40.0	
Gelatin	0.00				40.0
Sucrose	15.8	6.4	6.4	6.4	6.4
Starch	55.0	24.4	24.4	24.4	24.4
Codliver oil	6.0	6.0	6.0	6.0	6.0
Glucosamine hydrochloride	0.8	0.8	0.8	0.8	0.8
Cholesterol	0.5	0.5	0.5	0.5	0.5
Sodium citrate	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3
Vitamin mixture*	2.7	2.7	2.7	2.7	2.7
Mineral mixture**	8.6	8.6	8.6	8.6	8.6
Cellulose	6.5	6.5	6.5	6.5	6.5
Sodium carbonate	1.0	1.0	1.0	1.0	1.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Agar agar	2.0	2.0	2.0	2.0	2.0
Crude protein %	0.5	31.9	32.8	31.0	34.1

* Composition of vitamin mixture (g/100 g): Ascorbic acid 2.0, choline chloride 0.12, cyanocobalmine 0.00008, folic acid 0.08, nicotinic acid 0.04, pantothenic acid (calcium salt) 0.06, para aminobenzoic acid 0.01, pyridoxine hydrochloride 0.012, riboflavin 0.008, thiamine hydrochloride 0.004, biotin 0.00004, β -carotene 0.0096, calciferol 0.0012, inositol 0.2, menadione 0.004, and α -tocopherol 0.029.

** Composition of mineral mixture (g/100 g): Calcium lactate 2.72, potassium dihydrogenorthophosphate 2.0, sodium dihydrogen orthophosphate 0.79, magnesium sulphate 3.02, manganese chloride 0.004, and ferrous chloride 0.015.

Table 2. Composition of the purified diets PE₅ to PE₁₄

Ingredients	PE ₅	PE ₆	PE ₇	PE ₈	PE ₉	PE ₁₀	PE ₁₁	PE ₁₂	PE ₁₃	PE ₁₄
Albumen (egg)	20.0	30.0	40.0	50.0	60.0					
Casein (fat free)						20.0	30.0	40.0	50.0	60.0
Codliver oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Sucrose	11.0	8.6	5.8	3.0	0.4	11.0	8.6	5.8	3.0	0.4
Starch	44.4	33.2	22.2	11.4	0.4	44.4	33.2	22.2	11.4	0.4
Vitamin mix*	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Mineral mix**	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glucosamine HCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cellulose	0.9	4.5	8.2	11.9	15.5	0.9	4.5	8.2	11.9	15.5
Chromium oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium bicarbonate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Agar agar	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein %	16.8	24.8	33.3	41.3	51.5	17.7	29.3	37.4	48.4	53.4

*Vitamin and mineral mixtures were the same as in diets PE₀ to PE₄ (Table 1).

The solid ingredients of the diet were finely ground and homogenized thoroughly after addition of the codliver oil. The binder agar agar was dissolved in 40 ml of hot water (50–60°C) and added to the diet mixture. The diet was steamed for 10 minutes and extruded through a 3 mm diameter die. The pellets were dried at 60°C for 12 hrs and stored in a desiccator.

Feeding experiments

Hatchery-raised early juveniles of *P. indicus* were stocked in 10 litre capacity circular containers at the rate of eight animals per tank having filtered (through bolting cloth No. 30) brackish water of salinity $16.0 \pm 1\text{‰}$, temperature $28.5 \pm 0.1^\circ\text{C}$, pH 8.1 ± 0.1 and oxygen $4.0 \pm 0.1/\text{litre}$. There were three replicates for each treatment. The background of the bottom of the rearing tanks was specifically made milky white to facilitate easy recognition and collection of faeces for digestibility studies. The animals were fed twice a day in the morning and evening at the rate of 10% to 20% body weight. Leftover food was recovered, sediments removed, and three-fourths of the water replaced every day. The water was aerated intermittently with the help of an air blower. The duration of each feeding experiment was 30 days.

The faeces were collected carefully 3 hrs after the morning feeding (maximum shedding of faeces was observed to occur three hours after the feeding) with pipette on to a piece of bolting cloth. After the adhering salt was gently washed with distilled water, the faeces were transferred to a petri dish and dried in the oven at 60°C to constant weight. Collection of faeces was commenced only one week after the starting of the feeding experiment and continued till the end of the experiment. The design and planning of the feeding experiment simultaneously for digestibility studies and growth evaluation provided a longer period for faeces collection and to gather adequate quantity for chemical analysis.

Metabolic faecal nitrogen

The metabolic faecal nitrogen (MFN) was determined for the first time in the prawn *P. indicus* by feeding the animals with zero protein diet. For this purpose the animals were individually held separately in six rearing tanks and fed with zero protein diet PE₀ ad libitum for a period of 30 days. The faeces were collected every day as described earlier. The chromium oxide in the diet and faeces was determined spectrophotometrically using diphenyl carbazide reagent following the method of McGinnis and Kasting (1964). The MFN was calculated from the following formula:

$$\text{MFN excreted for 100 g of dry diet consumed} = \frac{A \times B}{C}$$

where A = % nitrogen in faeces of animals fed zero protein diet

B = % chromium oxide in zero protein diet

C = % chromium oxide in faeces

For calculating true digestibility, correction of faecal nitrogen of the diet was carried out as follows:

$$\text{MFN due to the amount of test diet consumed} = \frac{E}{D} \times \frac{AB}{C}$$

where D = % chromium oxide in test diet

E = % chromium oxide in the faeces of animals fed the test diet

The MFN thus obtained was subtracted from the total faecal nitrogen of the test group animals to obtain the corrected faecal nitrogen.

True digestibility of protein

True digestibility of protein was calculated using the formula

$$\text{TD (\%)} = 100 - \frac{\% \text{ Cr}_2\text{O}_3 \text{ in diet}}{\% \text{ Cr}_2\text{O}_3 \text{ in faeces}} \times \frac{\% \text{ corrected protein in faeces}}{\% \text{ protein in diet}} \times 100$$

Protein efficiency ratio

The PER was calculated by the formula

$$\text{PER} = \frac{\text{Average live-weight gain}}{\text{Average protein consumed}}$$

Net protein utilization

The NPU was determined using the modified formula of Castell and Tiews (1980) for fish:

$$\text{NPU} = \frac{\text{Body nitrogen of test group animals} - \text{Body nitrogen of animals fed with zero protein diet}}{\text{Nitrogen consumed}}$$

Biological value

The BV of the protein was calculated by the following formula:

$$BV = \frac{\text{Net protein utilization}}{\text{True digestibility}}$$

Total nitrogen in diets, animals, and faeces was determined by standard Kjeldahl method. The data obtained in the feeding experiments on various parameters were subjected to analysis of variance (ANOVA) following Snedecor and Cochran (1973). The means were compared by the least significant difference (LSD) test.

RESULTS

The excretion of nitrogen in the faeces and MFN of the prawns fed with zero protein diet are given in Table 3. There was variation in the nitrogen excreted in the faeces by the individual animals. The average value of nitrogen (N) excreted was 1.46% with a minimum of 1.04% and a maximum of 1.75%. The MFN varied from 248.5 mg to 351.6 mg per 100 g of diet consumed with an average of 326.4 mg per 100 g of diet.

Among the four purified proteins tested, gelatin was found to have the highest TD value of 93.41% ($p < 0.05$), followed by fibrin, with a value of 82.42% (Table 4). The TDs of albumen and casein were 72.48% and 76.44% respectively. But fibrin had shown the highest BV of 65 compared to that of albumen (59), casein (49), and gelatin (39). However, the differences were not found to be statistically significant ($p > 0.05$). The protein of fibrin had high NPU, followed by albumen, casein, and gelatin.

The results with the zero protein diet were interesting. The prawns fed with this diet had lost 5.4% in live-weight and 4.8% in dry weight. Gradually, the animals became less active and eventually died. At the end of the experiment the body nitrogen of the prawns fed with this diet came down to 9.91% from an average initial value of 10.94% and the corresponding loss of body protein was 6.43%. The prawns fed with the albumen diets grew significantly ($p < 0.01$) faster with good FCR ($p < 0.05$) and 100% survival. On the other hand, the growth of prawns fed

Table 3. Metabolic faecal nitrogen (MFN) in juvenile *P. indicus* determined using zero protein diet

Experiment no.	Chromium oxide %		Nitrogen in faeces, %	Metabolic faecal nitrogen (mg of N per 100 g diet consumed)
	in diet	in faeces		
1	0.4342	1.8169	1.40	334.6
2	0.4342	1.8169	1.40	248.5
3	0.4342	1.8167	1.43	341.8
4	0.4342	2.1500	1.72	347.4
5	0.4342	2.1610	1.75	351.6
6	0.4342	1.8167	1.40	334.6
Average		1.9297	1.46	326.4

Table 4. Data of the feeding experiment conducted with diets PE₀ to PE₄ on juvenile *P. indicus* for 30 days

Parameter	PE ₀	PE ₁	PE ₂	PE ₃	PE ₄
True digestibility of protein (TDP)		72.48 ^a	76.44 ^b	82.42 ^c	93.41 ^d
Protein efficiency ratio (PER)		1.34 ^a	0.76 ^b	0.67 ^b	0.27 ^c
Net protein utilization (NPU)		43.0	36.0	53.0	38.0
Biological value (BV)		59.0	49.0	65.0	39.0
Growth in length %	Nil	52.1 ^a	33.2 ^b	27.0 ^c	20.6 ^d
Growth in live-weight %	-5.4	359.4 ^a	191.6 ^b	214.3 ^b	118.8 ^{bc}
Growth in dry weight %	-4.8	298.5 ^a	135.7 ^b	184.6 ^c	64.1 ^d
Food conversion ratio (FCR)		2.40 ^a	4.17 ^b	5.08 ^{bc}	9.82 ^d
Survival %	60.0	100.0	53.3	100.0	53.0

Values with the same superscripts in the same row do not differ significantly among themselves. TDP, PER, growth in length, growth in dry weight and FCR were significant at 1% ($p < 0.01$) and growth in live-weight was significant at 5% ($p < 0.05$). NPU and BV were not significant at 5% ($p > 0.05$).

with gelatin diet was significantly low and produced high FCR and poor survival. Again the albumen diet only recorded high PER followed by casein and gelatin diets ($p < 0.01$). The growth, FCR, and PER obtained by casein and fibrin diets were comparable.

The growth of prawns increased with the increase in the dietary protein level up to a certain value with both albumen and casein diets (Table 5). In the case of albumen diets maximum growth ($p < 0.01$) was recorded with the diet having 25% protein, whereas in the case of casein diets it was at 29.3% protein. The growth declined with further increase in the dietary protein level in both cases. Similarly, at these two respective levels of protein in albumen and casein diets, high PER and low FCR were achieved. The TD of protein tended to be higher at lower dietary protein levels (Fig. 1a) but decreased at 33.3% protein in albumen diets and at 37.4% in casein diets. And it again increased at higher dietary protein levels. The NPU and BV increased (Fig. 1b, c) with increase in the dietary protein level and recorded a peak at 33.3% protein in albumen diets and at 29.3% protein in casein diets. Both the parameters declined with further increase in the dietary protein level. While the differences in TD, NPU, and BV at different protein levels of albumen diets were not significant ($p > 0.05$), those at different protein levels of casein diets were statistically significant ($p < 0.01$).

Dietary protein level and excretion of faecal nitrogen

The relationship between the dietary protein level and faecal nitrogen (FN) excreted per 100 g of diet consumed is shown in Fig. 2. The FN rapidly increased from 326.4 mg to 2667.5 mg with the dietary protein level and registered a peak at 25% dietary protein in albumen diets and at 37% protein level in casein diets. In both the cases, the FN rapidly declined from 2667.5 mg to 706.6 mg with further increase in the dietary protein level. It is amply clear that the excretion of nitrogen

Table 5. Data of the feeding experiments conducted with diets PE₅ to PE₁₄ on juvenile *P. indicus* for 30 days

Parameter	PE ₅	PE ₆	PE ₇	PE ₈	PE ₉	PE ₁₀	PE ₁₁	PE ₁₂	PE ₁₃	PE ₁₄
TDP	87.34	91.43	79.35	91.72	96.64	92.45 ^a	95.05 ^a	79.15 ^b	98.52 ^a	97.37 ^a
PER	0.60 ^b	1.16 ^a	0.43 ^b	0.46 ^b	0.56 ^b	0.65 ^a	0.92 ^a	0.27 ^c	0.19 ^c	0.34 ^{abc}
NPU	33.90	45.86	47.89	44.70	38.71	47.13 ^a	66.69 ^b	43.49 ^a	28.68 ^c	51.68 ^d
BV	33.81	50.16	59.72	48.74	40.06	50.93 ^a	70.33 ^b	55.33 ^{ca}	299.67 ^a	53.00 ^{ac}
Growth in length %	32.2 ^b	59.1 ^a	32.0 ^b	32.3 ^b	34.6 ^c	45.2 ^a	48.8 ^a	36.1 ^b	24.1 ^c	28.0 ^{bc}
Growth in live-weight %	146.7 ^b	256.6 ^a	138.7 ^b	148.8 ^b	126.3 ^b	179.5 ^a	257.5 ^b	137.4 ^{ac}	90.1 ^{cd}	111.4 ^{cd}
Growth in dry weight %	154.5 ^b	262.7 ^a	154.2 ^b	155.8 ^b	167.1 ^b	121.7 ^a	238.4 ^b	106.5 ^{ac}	81.7 ^{cd}	93.6 ^{cd}
FCR	6.15	3.49	8.49	8.47	3.63	7.89 ^a	5.96 ^{ab}	9.92 ^c	10.67 ^c	5.50 ^{bd}
Survival %	45.8	75.0	75.0	50.0	58.3	66.0	62.5	50.0	45.8	58.0

Values with different superscripts in the same row differ significantly among themselves. The growth in length, live-weight, and dry weight for the diets PE₅ to PE₉ were significant at 1% ($p < 0.01$) and their TDP, NPU, BV, and FCR were not significant ($p > 0.05$). Similarly, the growth in length, dry weight, TDP, PER, NPU, and BV of diets PE₁₀ to PE₁₄ were significant at 1% ($p < 0.01$) and their live-weight and FCR were significant at 5% ($p < 0.05$).

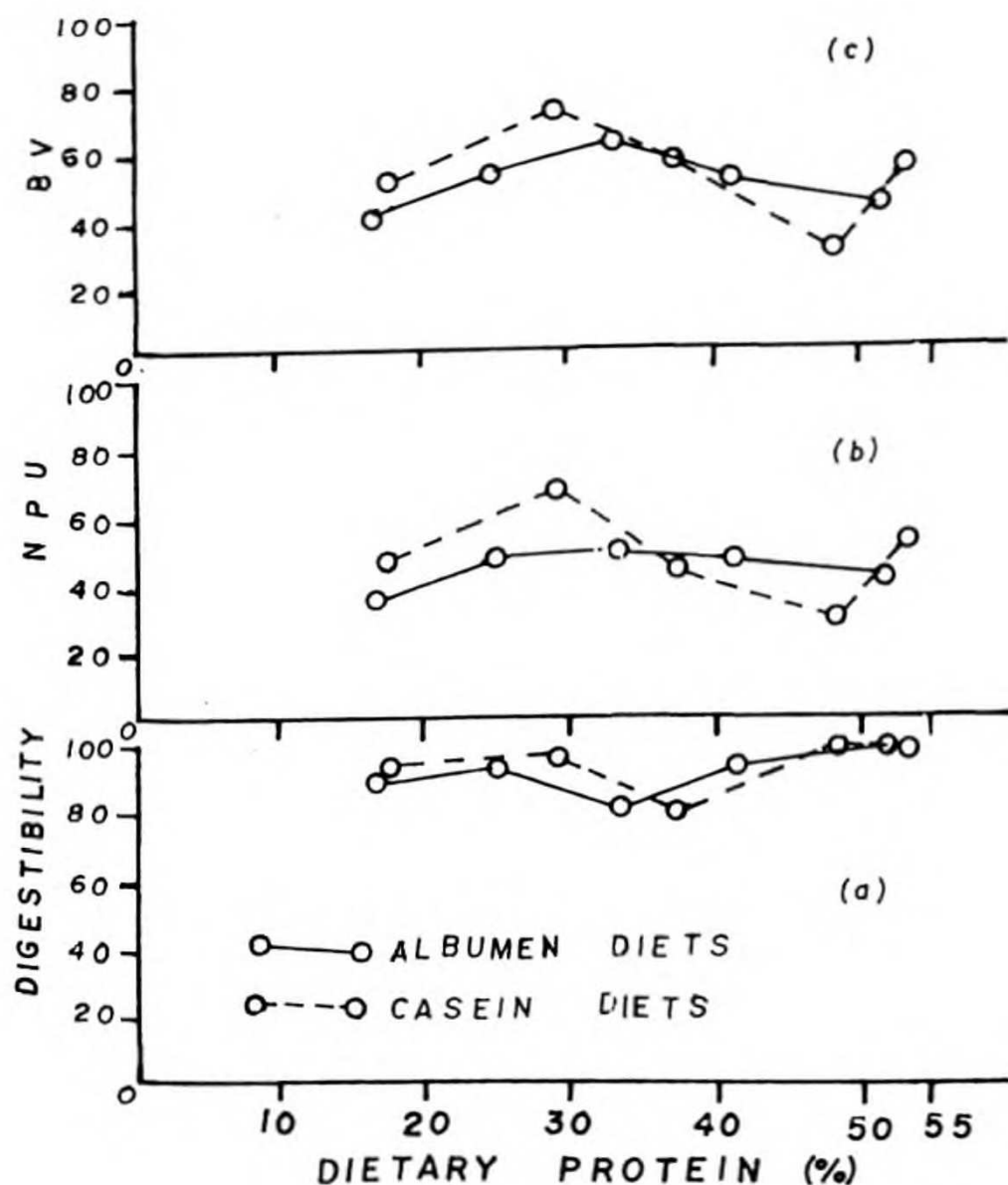


Fig. 1. Influence of dietary protein level on (a) digestibility, (b) NPU, and (c) BV in juvenile *P. indicus*

was higher in the prawns fed with albumen diets than in those fed with casein diets.

Nitrogen balance

The nitrogen balance was calculated as the difference in the nitrogen of the diet and the nitrogen in the faeces of the animals fed with that diet. The relationship between the dietary protein and the nitrogen balance is depicted in Fig. 3. It is interesting to note that at lower dietary protein levels, the nitrogen balance is negative and as the protein in the diet increases the nitrogen balance becomes positive and gradually increases. The results are similar with both albumen and casein diets. In both cases the nitrogen balance curve intercepts the protein axis

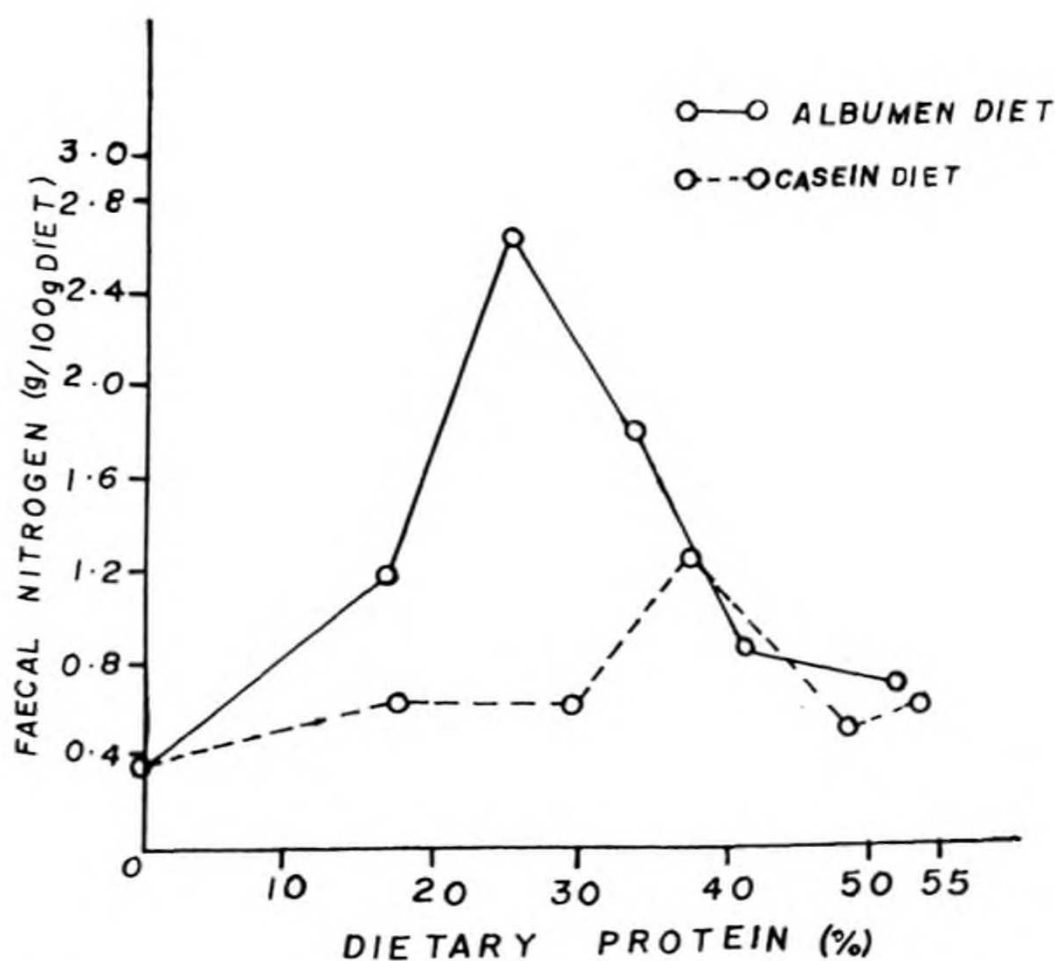


Fig. 2. Relationship between dietary protein and faecal nitrogen in juvenile *P. indicus*

(y-axis) at 22.5% protein. At this protein level, the nitrogen balance is zero, below which it is negative and above which it is positive.

DISCUSSION

The MFN, determined for the first time in *P. indicus*, varied from 248.5 mg to 351.6 mg per 100 g of dry diet consumed. Studies on MFN in certain fishes (Nose, 1967; Ogino and Chen, 1973a; Jauncey, 1982), prawn (Forster and Gabbott, 1971), albino rats, humans (Mitchell and Bert, 1954), and ruminants and non-ruminants (Maynard *et al.*, 1981) have shown that the values varied from animal to animal. Differences were also found in the values of MFN within the same species determined by different workers as in the case of carp. In *P. indicus*, the MFN is found to be greater than the values reported for *Palaemon serratus* (Forster and Gabbott, 1971) and very much less than the values reported in ruminants. Besides the digestive juices, epithelial cells abraded from the walls of the alimentary canal and the residual contents from the bacteria contribute

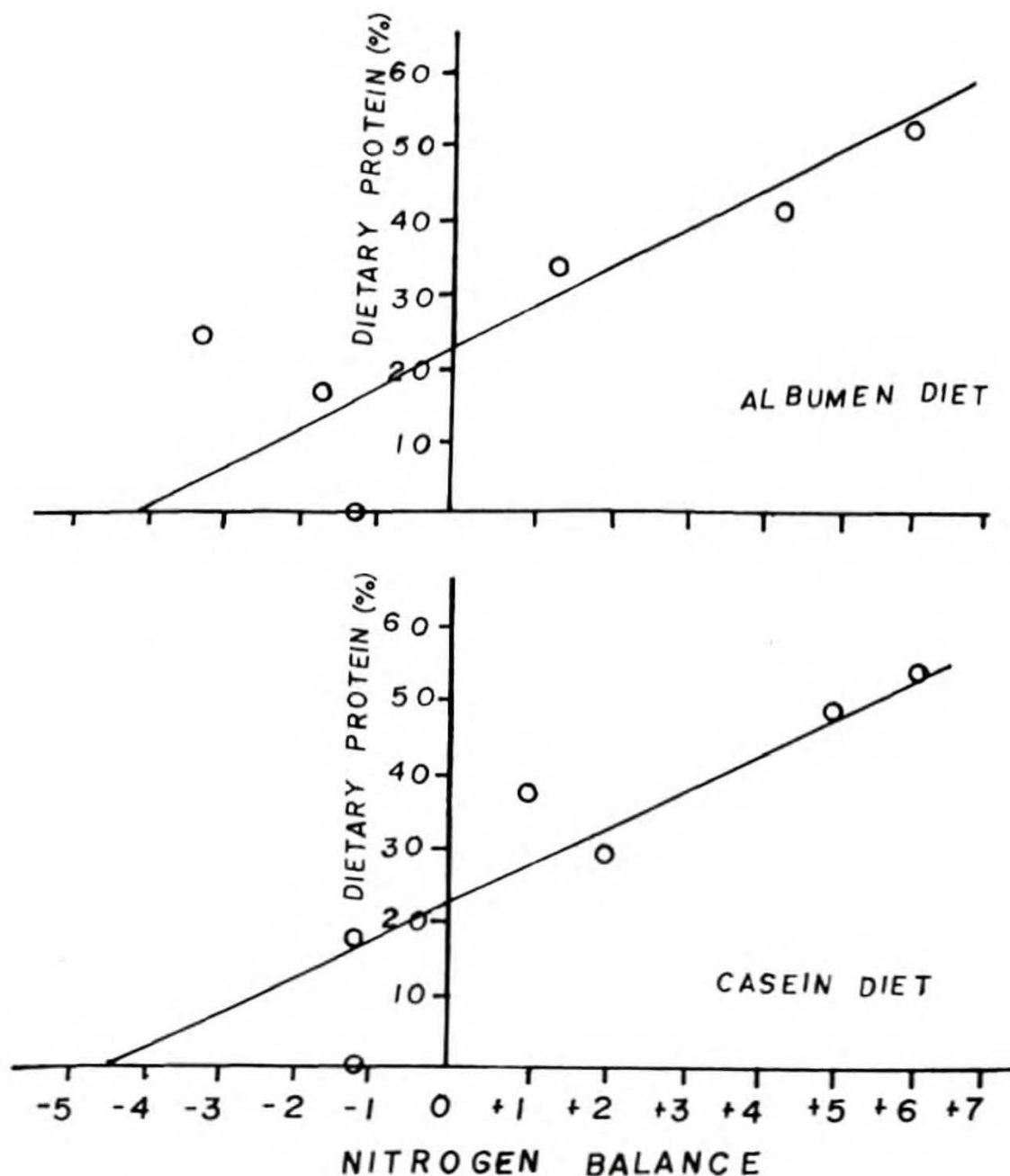


Fig. 3. Relationship between dietary protein and nitrogen balance in juvenile *P. indicus*

to MFN. Prawns are known to secrete a chitinous peritropic membrane round the faecal pellets (Forster, 1953). The higher value of MFN in prawns in general among the aquatic animals might be due to this peritropic membrane in the faeces. The difference in the MFN between the prawns *P. indicus* and *P. serratus* is significant and might be due to the differences in the nature and quantity of the faecal membrane in the two types of prawns. Determination of MFN in more species of penaeid and the caridean prawns might throw more light on this aspect.

The TD of albumen was low and that of gelatin was high. Forster and Gabbott (1971) showed that the assimilation of albumen was 96.6% in *P. serratus* and

Pandalus platyceros and the TD of casein as well as gelatin was 97.7%. Recently, Akiyama *et al.* (1989) have determined the apparent digestibility of casein and gelatin in *Penaeus vannamei* and reported that it was 99.1% for casein and 97.3% for gelatin. While the digestibility of casein in *P. indicus* is lower than that found in the caridean prawns and *P. vannamei*, the digestibility of gelatin in all the prawns is practically comparable. These differences might be due to the difference in protein levels used in the diets as Forster and Gabbott (1971) used 60% and Akiyama *et al.* (1989) used 90%, whereas we used only 40% in the present study. In the subsequent experiments, it was found that when the diets contained 60% protein, the TDs of albumen and casein were 96% and 97% respectively, which were comparable to the figures reported for the caridean prawns. Although Forster and Gabbott (1971) observed that the influence of MFN on determining the TD of protein is related to the dietary protein level, the effect of MFN is more marked on the TD at lower dietary protein level and its influence is negligibly small on the TD at higher levels of protein in the diet. This is due to the fact that at lower dietary protein levels, the constant excretion of MFN makes up a greater proportion of the faecal nitrogen output which becomes progressively less as the dietary protein level increases (Maynard *et al.*, 1981).

Gelatin, which showed low PER, gave NPU comparable to that of casein. But the BV of gelatin came down because of low PER and high TD. This is also due to the fact that gelatin is deficient in essential amino acids except arginine. The BV of fibrin was superior to that of albumen and casein. Data on NPU and BV of purified proteins for prawns are scarce, but they are determined in some finfishes (Attack and Matty, 1978; Teshima *et al.*, 1978). While the NPU and BV obtained in *P. indicus* were comparable to those obtained in carp and rainbow trout, NPU of casein in *P. indicus* was superior to the value reported in tilapia. On the other hand, the NPU and BV of albumen and fibrin obtained in *P. indicus* were superior to the NPU and BV of casein in finfishes. However, the values of NPU and BV of gelatin in this prawn are inferior to those obtained in carp and rainbow trout using casein diets. Thus, albumen and fibrin have high BVs followed by casein for this prawn. Considering the growth of prawns, FCR and PER albumen appears to be a better protein source among the four purified protein sources tested.

Protein requirement in the diet of penaeid prawns showed wide variations (from 20% to 57%) not only between two different species but also within the same species as determined by different workers using different protein sources (Shewbart *et al.*, 1973; Shigueno *et al.*, 1972; Venkataramaiah *et al.*, 1975; Zein-Eldin and Corliss, 1976; Colvin, 1976; Deshimaru and Yone, 1978; Ahamad Ali, 1982). In most of these studies the protein requirement was determined by measuring the growth, FCR and in some cases PER as a response to the dietary protein level. These studies were summarized by Kanazawa (1984 and 1989) and the differences were explained as due to the differences in the amino acid composition of protein sources, especially of the essential amino acids, used for formulating the diets.

To understand the intricacies in the protein requirement, the effect of protein level in the diet on its digestibility, PER, NPU, and BV, in conjunction with growth and FCR was investigated using two different protein sources. This approach had

yielded interesting results. With albumen diets the prawn had shown a protein requirement of 24.7% and with casein diets the requirement shown was 29.3%. The difference in the protein requirement shown by the two protein sources is 4.55% and can be considered an important difference. Though the TD of protein did not show any clear trend with dietary protein level, it tended to be high at higher levels and low at lower levels of protein in the diet. Similar observations were made by Smith *et al.* (1985) in *P. vannamei*. As in the case of growth, the PER, NPU, and BV registered a peak at the same protein level and showed a decreasing tendency as the protein level in the diet increased.

The nitrogen excretion in faeces (Fig. 2) registered a peak at a particular protein level in the diet and declined thereafter. The results were similar in both albumen and casein diets. However, the peak of N excretion in faeces was at a higher level of dietary protein in casein diets (37.3%) than in albumen diets (24.6%). Does this not indicate that the nitrogen required for basal metabolism can be met at a lower dietary protein level with albumen diets and it could be met only at a higher level with casein diets? These findings show that the intraspecies differences in dietary protein requirement could be mainly due to the differences in the protein sources used.

In the present study PER, NPU, and BV decreased with increase in the dietary protein level. Similar trends were experienced in finfish and also in higher animals (Ogino and Saito, 1970; Ogino Chen, 1973a, b in carp; Teshima *et al.*, 1978 in *Tilapia zilli*; Jauncey, 1982 in *Sarotherodon mossambicus*; Albanis, 1972 in albino rats).

The protein requirement of *P. indicus* determined using albumen and casein diets in the present study is lower than the protein requirement of 42–43% determined for this prawn by Colvin (1976) and Ahamad Ali (1982) and also of many of the penaeid prawns. However, such results are not uncommon. New (1976), reviewing earlier works, concluded that the protein requirement in the diet of penaeid prawns lies between 27% and 35%. In this context, the protein balance (Fig. 3) worked out in the present study offered good explanation for understanding such results of low protein requirement. The nitrogen balance curve intercepts the protein axis at 22% of dietary protein in both albumen and casein diets. At this point, the intake and excretion of protein in faeces are the same. A small increase in the protein (2.75% in case of albumen diets and 7.3% in casein diets) of the diet above this resulted in the highest growth and protein deposition in the body of prawns. Any further increase in the dietary protein, though it resulted in positive nitrogen balance, only decreased the growth and protein deposition in the body of prawns.

From these findings it is clear that the dietary protein required to achieve the highest growth and the best FCR is just above the dietary protein at which the nitrogen balance is zero. This also explains the low values of protein requirement shown by *P. indicus* in this study. Here it should be emphasized that the method of nitrogen balance could play an important role in determining the protein requirement of aquatic animals more accurately. It is therefore recommended that nitrogen balance should be investigated along with the determination of growth, FCR, and PER for fixing the optimum protein level more precisely. It is well known

that protein contributes towards the major portion of the cost of the feed, and determination of nitrogen balance along with other parameters might greatly help in keeping protein levels at the minimum leading to more economical practical feed formulation.

In conclusion it can be said that all the purified proteins do not have the same rating of biological quality. It is necessary to use protein source with good BV for formulation of research diets for protein requirement studies. Albumen (egg) seems to be a better protein source for this purpose.

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